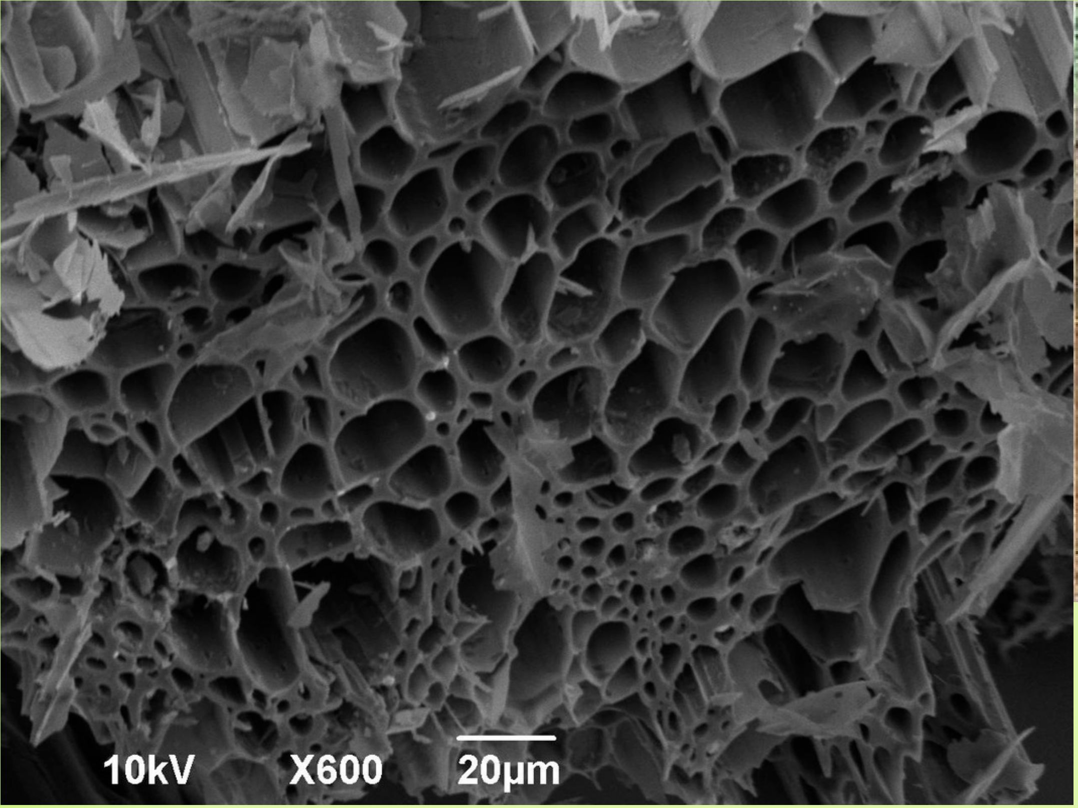

http://www.google.com/search?q=biochar&tbm=isch&tbo=u&source=univ&sa=X&ei=iJq3UcfvMarxygGA1oC4Cw&sqi=2&ved=0CEEQsAQ&biw=1280&bih=601#imgrc=_

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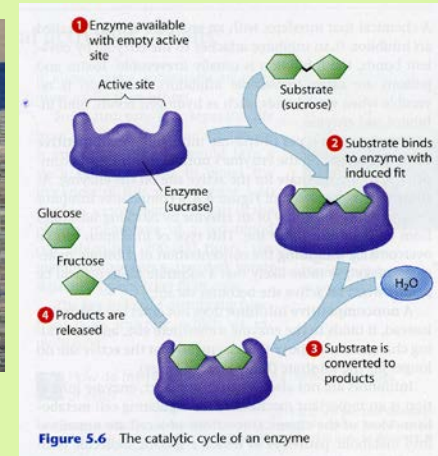
http://www.biochar.org/joomla/images/stories/OkimoriBiochar.jpg

http://www.treehugger.com/natural-sciences/reforestation-biochar-two-geoengineering-methods-that-wont-cause-more-harm-than-good.html

Long Term Effects of Biochar on Select Soil Enzyme Activities



http://www.greencityltd.com/enzymescience.html

M. R. Bayan, PhD, PhD
Environmental Geochemist and Soil Scientist
Lincoln University
Soil and Environmental Sciences

Photo: M. R. Bayan, Lincoln University, MO

Forward

"Nutrient cycling in soils involves biochemical, chemical, and physiochemical reactions, with the biochemical processes being mediated by microorganisms, plant roots, and soil animals."

"It is well known that all biochemical reactions are catalyzed by enzymes, which are proteins with catalytic properties owing to their power of specific activations."

Tabatabai, M. A. (1994). Soil Enzyme *in*: Methods of Soil Analysis, part 2. Microbiological and Biochemical Properties – SSSA Book Series, no. 5

Forward

"Enzymes are catalysts, that is, they are substances that without undergoing permanent alteration, cause chemical reactions to proceed at faster rates."

"In addition, they are specific for the types of chemical reactions in which they participate."

Tabatabai, M. A. (1994). Soil Enzyme *in*: Methods of Soil Analysis, part 2. Microbiological and Biochemical Properties – SSSA Book Series, no. 5

Forward

"Enzymes are denatured by elevated temperature and extreme pH. Their physiochemical state and their influence on chemical reactions are markedly dependent on pH, ionic strength, temperature, and the presence or absence of inhibitors or activators."

Tabatabai, M. A. (1994). Soil Enzyme *in*: Methods of Soil Analysis, part 2. Microbiological and Biochemical Properties – SSSA Book Series, no. 5

Soil Enzyme Activities in the Presence of Biochar?

Knowledge about how and why biochar works is being generated at an accelerating rate. However, little is known about the long term effect of biochar on soil enzymes

This knowledge is necessary because an understanding of biochar effects on soil enzyme activities provides information about its effects on nutrient cycling in the pedosphere.

Samples of Previous Work on the Effect of Biochar on Soil Enzyme Activities

"…biochar chemically altered or blocked the binding site of the substrate"

Fansler et al., 2009

http://scisoc.confex.com/crops/2009am/webprogram/Paper55515.html

When biochar present, "The activities of cellulase, urine enzyme, neutral phosphatase and sucrase were also found to increase by 117.4–178.3 %, 31.1–37.6 %, 29.7–193.8 % and 36.5–328.6 %, respectively."

Yang et al., 2013

http://www.biochar-international.org/node/3838

Objectives of this Study

1. How does the presence of biochar in soil affect the activities of soil enzymes, acid- and alkaline-phosphatase, arylamidase and β-glucosidase?
2. How do biochars produced from different precursors (ligneous, pine and herbaceous, miscanthus) affect the activities of these enzymes?
3. Does the rate of applied biochar affect enzyme activities?

Phosphomonoestrases: Acid- and Alkaline-Phosphatases

What do theses enzymes do in the soil environment?

They "hydrolyse monoester bonds (including mononucleotides and sugar phosphates), phosphoprotein phosphatases (which hydrolyse phosphoester bonds of phosphoserines, phosphothreonines or phosphotyrosines), phytases (EC 3.1.3.26 for 4-phytase and EC 3.1.3.8 for 3-phytase, which hydrolyse all six phosphate groups from inositol hexaphosphate) and nucleotidases.."

Nannipieri et al. (2011). Role of Phosphatase Enzymes in Soil
http://www.bashanfoundation.org/paolo/paoloenzymes.pdf

Arylamidase [α-aminoacyl-peptide hydrolase (microsomal) EC 3.4.11.2]

What does it do in the soil environment?

It "catalyzes the release of an N-terminal amino acid from peptides, amides, or arylamides. Because of the presence of such substrates in soils, it is likely that this enzyme is involved in N mineralization."

Acosta-Mart and Tabatabai (2000). Arylamidase Activity of Soils
https://www.soils.org/publications/sssaj/abstracts/64/1/215?access=0&view=pdf

β -Glucosidase

What does this enzyme do in the soil environment?

"… A group of enzymes that catalyze the hydrolysis of different glycosides. The general equation of the reaction is: Glycosides + H_2O → Sugar + Aglycones. …The hydrolysis products of β-glucosidases are believed to be important energy sources for microorganisms in soils. Its activity is correlated with the organic C content of surface soils and of soil profiles"

Tabatabai, M. A. (1994). Soil Enzymes. In: Methods of Soil Analysis – Part 2, Microbiological and Biochemical Properties. Editors: Weaver et al. (1994)

The Soil

A cultivated Alfisol, 5 kg

CEC = 12.0 meq/100g

Neutralizable Acidity = 4.0 meq/100g

pH_s = 5.48

OM = 2.9%

P = 21.3	kg/ha	(19 lbs/A)	(Low)
K = 285	kg/ha	(254 lbs/A)	(High)
Ca = 2090	kg/ha	(1865 lbs/A)	(Medium)
Mg = 808	kg/ha	(721 lbs/A)	(Very high)

S (SO_4-S) = 2.7 ppm (Medium)

Greenhouse Experiment Treatments

Treatments were as follows:

No Biochar – No Soybean (*Glycine max*)

No Biochar – Soybean

2% Miscanthus Biochar (wt) – Soybean

5% Miscanthus Biochar (wt) – Soybean

2% Pine Biochar (wt) – Soybean

5% Pine Biochar (wt) - Soybean

All treatments were triplicated

Greenhouse Experiment

Soil samples were taken from each pot after 1-day, 3-day, 10-day, 30-day and 60-day intervals

All pots were treated with 75 kg P per hectare.

Additional data from a similar experiment with switchgrass will be used here for comparison.

Experiment duration: 60 days

Acid Phosphatase Assay
Eivazi and Tabatabai (1977)
Procedure:

Step 1: Weigh 1 gram of soil into a 50-mL Erlenmeyer flask.
Step 2: Add 0.2 mL Toluene.
Step 3: Add 4 mL of MUB (pH 6.5).
Step 4: Add 1 mL p-nitrophenyl phosphate (pH 6.5)
DO NOT ADD TO CONTROL.
Step 5: Swirl and stopper flask and incubate at 37° C for 1 hour
Step 6: Add 1 mL p-nitrophenyl phosphate (pH 6.5) to control.
IMMEDIATLY add 1 mL of 0.5M $CaCl_2$ and 4 mL of 0.5M NaOH, in THAT ORDER to each flask (swirl to mix).
Step 7: Filter through Whatman #2 filter paper.
Step 8: Pour into cuvette.
Step 9: Measure at 410nm; Dilute with deionized water if necessary.
Step 10: A standard curve will be generated from standard solutions containing 0, 10, 20, 30, 40, and 50 µg of *p*-nitrophenol.

Alkaline Phosphatase Assay
Eivazi and Tabatabai (1977)
Procedure:

Step 1: Weigh 1 gram of soil into a 50-mL Erlenmeyer flask.

Step 2: Add 0.2 mL Toluene.

Step 3: Add 4 mL of MUB (pH 11).

Step 4: Add 1 mL p-nitrophenyl phosphate (pH 11) DO NOT ADD TO CONTROL.

Step 5: Swirl and stopper flask and incubate at 37° C for 1 hour.

Step 6: Add 1 mL p-nitrophenyl phosphate (pH 11) to Control and IMMEDIATLY add 1 mL of 0.5M $CaCl_2$ and 4 mL of 0.5M NaOH in THAT ORDER. (swirl to mix).

Step 7: Filter through Whatman #2 filter paper.

Step 8: Pour into cuvette.

Step 9: Measure at 410nm; Dilute with deionized water if necessary.

Step 10: A standard curve will be generated from standard solutions containing 0, 10, 20, 30, 40, and 50 µg of *p*-nitrophenol.

Arylamidase Assay
Acosta-Martinez and Tabatabai (2000)
Procedure:

Step 1: Weigh 1 gram of soil in 25-mL Erlenmeyer flask.
Step 2: Add 3 mL of 0.1M THAM buffer (pH8).
Step 3: Add 1 mL of 8.0mM L-leucine β-naphthylamide hydrochloride DO NOT ADD TO CONTROL.
Step 4: Swirl and stopper the flask and incubate at 37° C for 1 hour.
Step 5: Add 1 mL of 8.0mM L-leucine β-naphthylamide hydrochloride to Control, IMMEDIATLY Add 6 mL of ethanol (95%) to each flask (swirl to mix)
Step 6: Pour into centrifuge tubes and centrifuge for 1min at 17000 x g.
Step 7: Transfer 1 mL of liquid to new tubes.
Step 8: Add 1 mL of ethanol, 2 mL of acidified ethanol, and 2 mL of p-dimethylaminocinnameldehyde mixing in vortex mixer after adding each reactant. Pour into cuvette.
Step 9: Measure at 540nm; dilute with ethanol if necessary
Step 10: A standard curve will be generated using 5, 10, 15, 20, 25, and 30 μg of β-naphthylamine/mL.

β-Glucosidase Assay
Eivazi and Tabatabai (1988)
Procedure:

Step 1: Weigh 1 gram of soil in 25-mL Erlenmeyer flask.
Step 2: Add 0.25 mL toluene.
Step 3: Add 4.0 mL MUB (pH 6.0). 4-methylumbelliferone (MUB)
Step 4: Add 1 mL PNG solution.
DO NOT ADD TO CONTROL
Step 5: Swirl to mix contents, stopper each flask, and incubate at 37° C for 1 hour.
Step 6: Add 1 mL PNG solution to control. IMMEDIATLY Add 1 mL of 0.5M $CaCl_2$ & 4 mL 0.1M THAM buffer (pH12) in THAT ORDER to each flask (swirl to mix).
Step 7: Swirl flask and filter through Whatman no. 2v folded filter Paper. Pour into cuvette.
Step 8: Measure absorbance on spectrometer at 410nm; dilute with 0.1M THAM (pH10) if reading is too high.
Step 9: A standard curve will be generated from standard solutions containing 0, 10, 20, 30, 40, and 50 µg of *p*-nitrophenol.

How Do We Produce Biochar?

Biochar – Byproduct of Bioenergy Production from Lignocellulosic Biomass

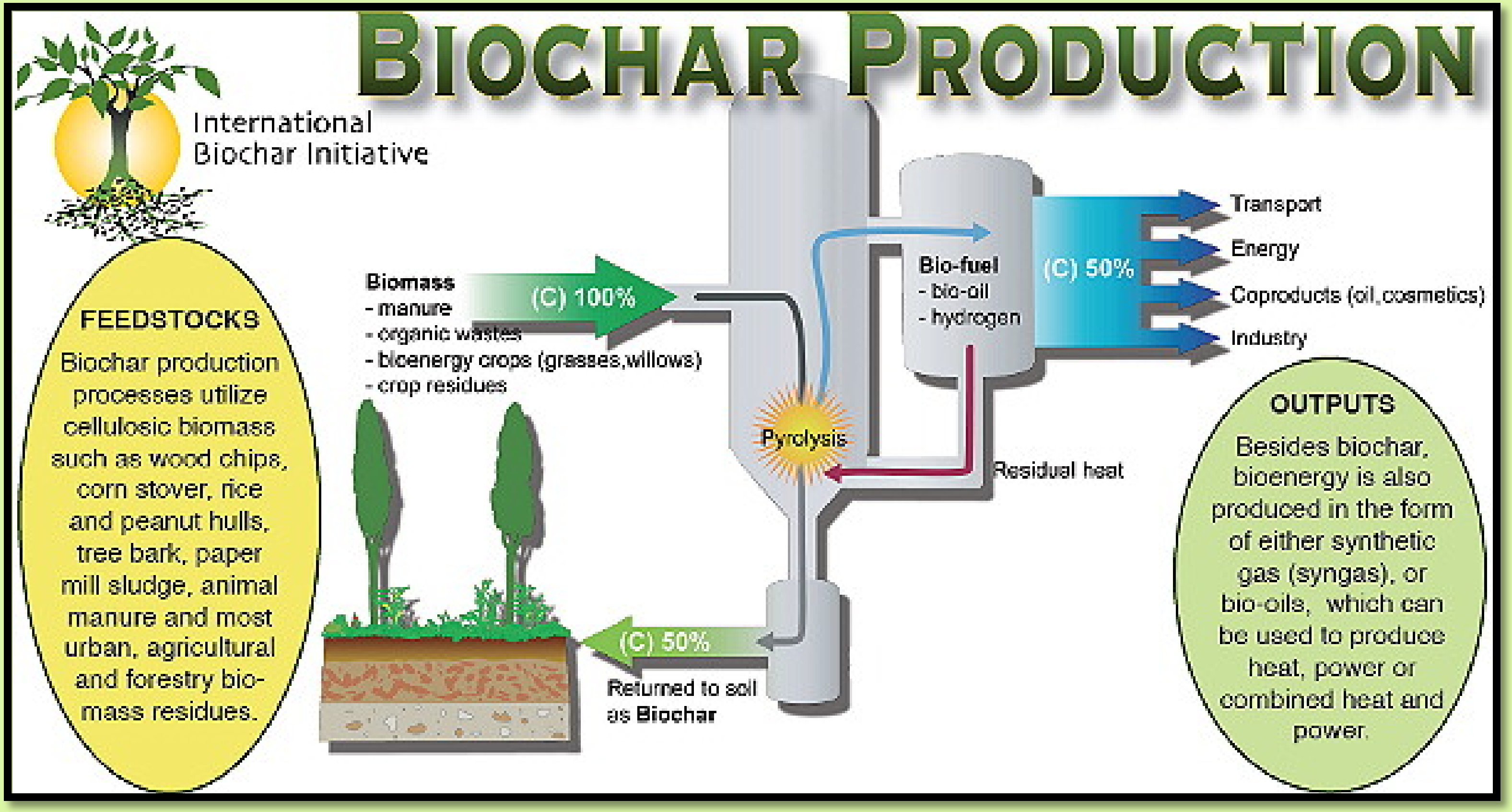


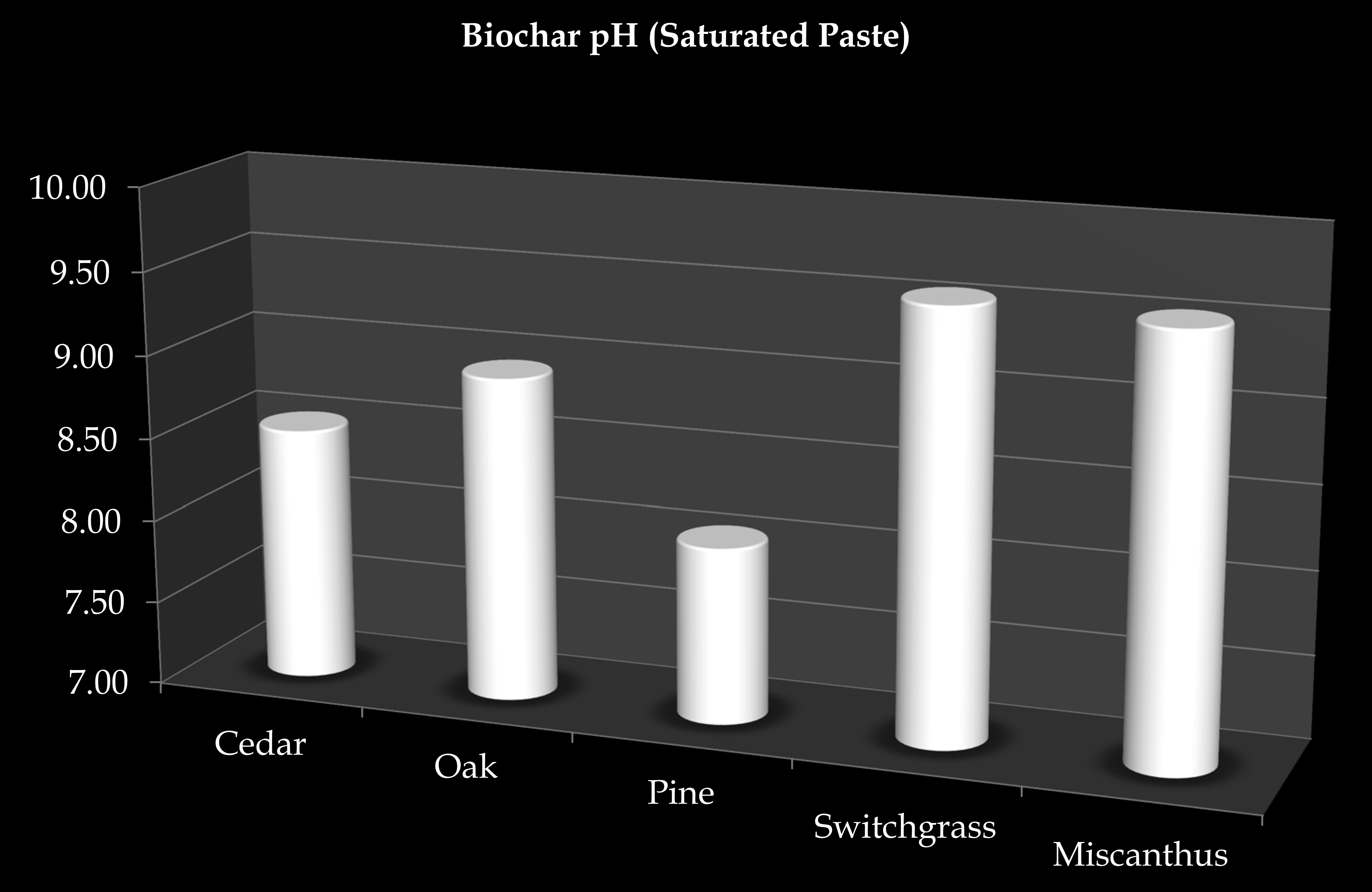
Photo courtesy of International Biochar Initiative

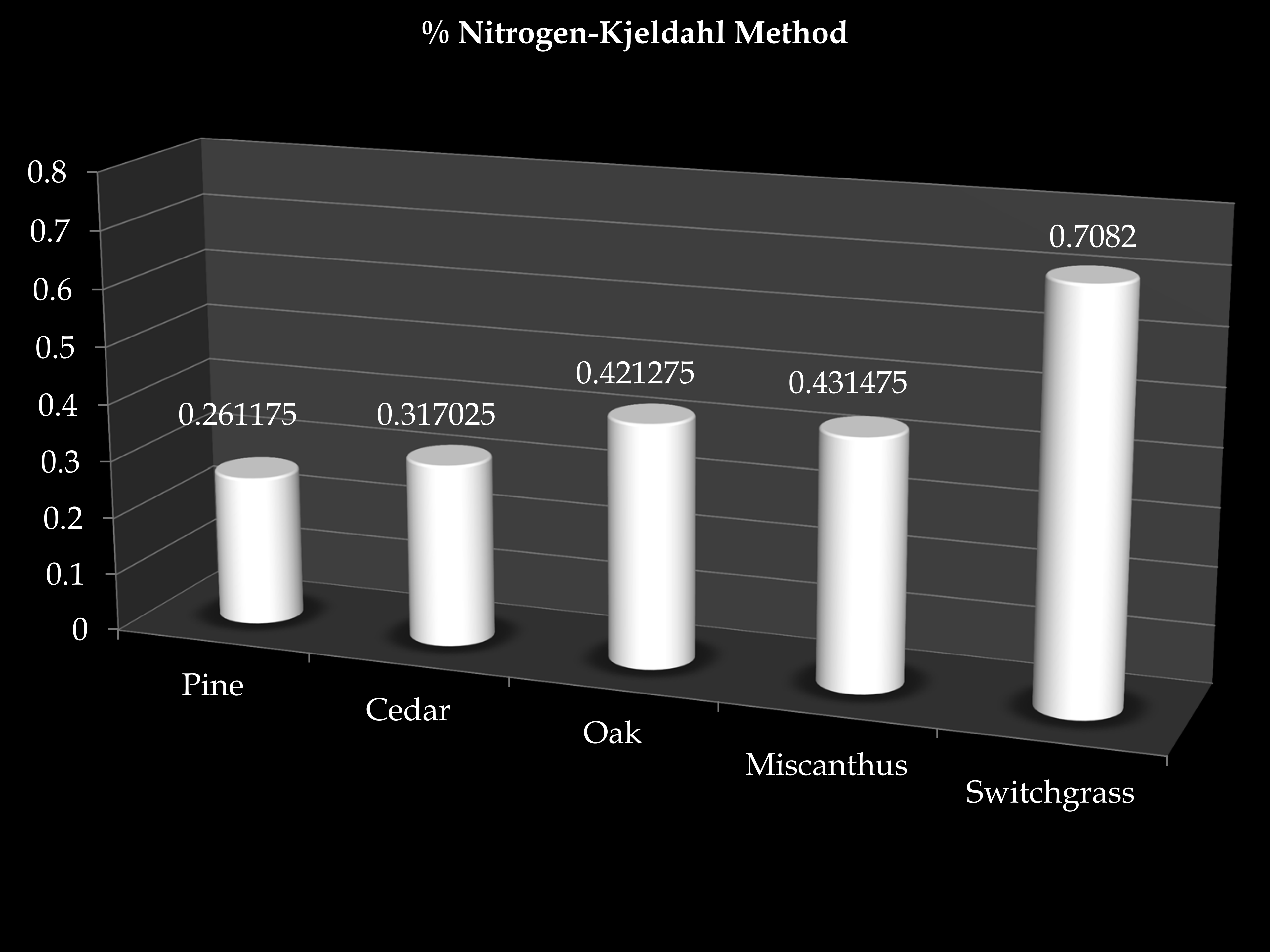
Biochar was Generated from a Variety of Feedstocks through Slow Pyrolysis


EXTECH
5 18.0
DATALOGGER
ENTER
TIME

Results and Discussion

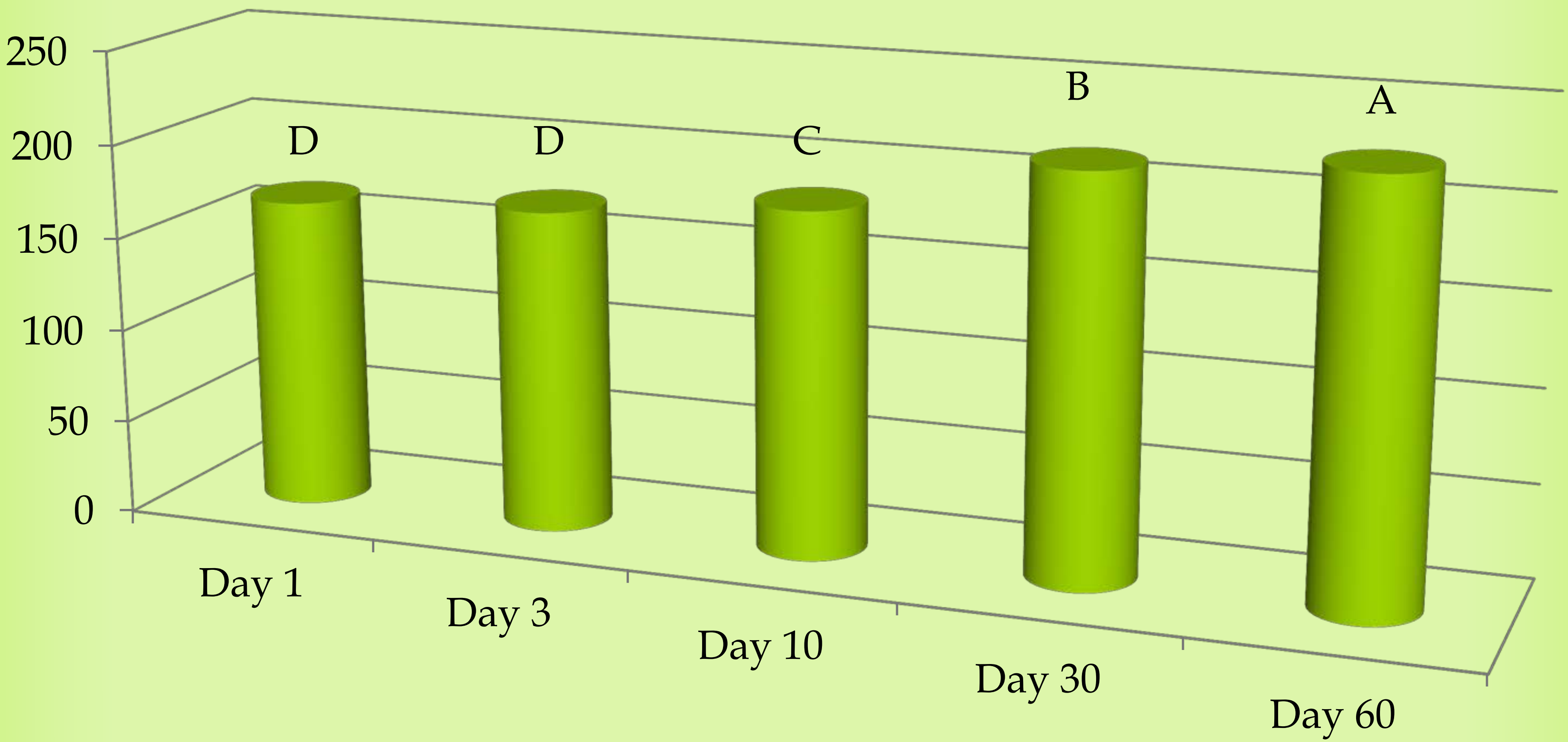
“Boy! I would love to be his pet cat.”


Biochar pH (Saturated Paste)
10.00
9.50
9.00
8.50
8.00
7.50
7.00
Cedar
Oak
Pine
Switchgrass
Miscanthus

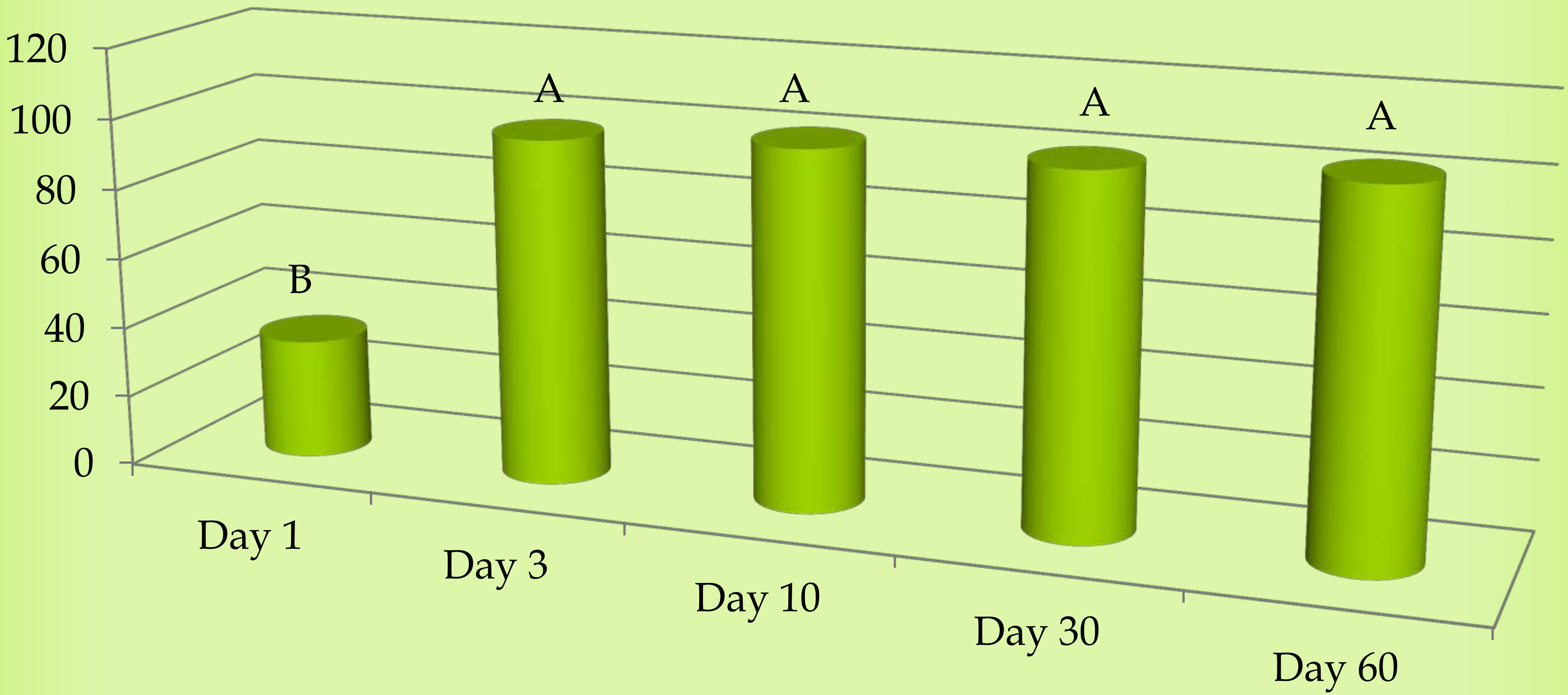

% Nitrogen-Kjeldahl Method
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.261175
0.317025
0.421275
0.431475
0.7082
Pine
Cedar
Oak
Miscanthus
Switchgrass

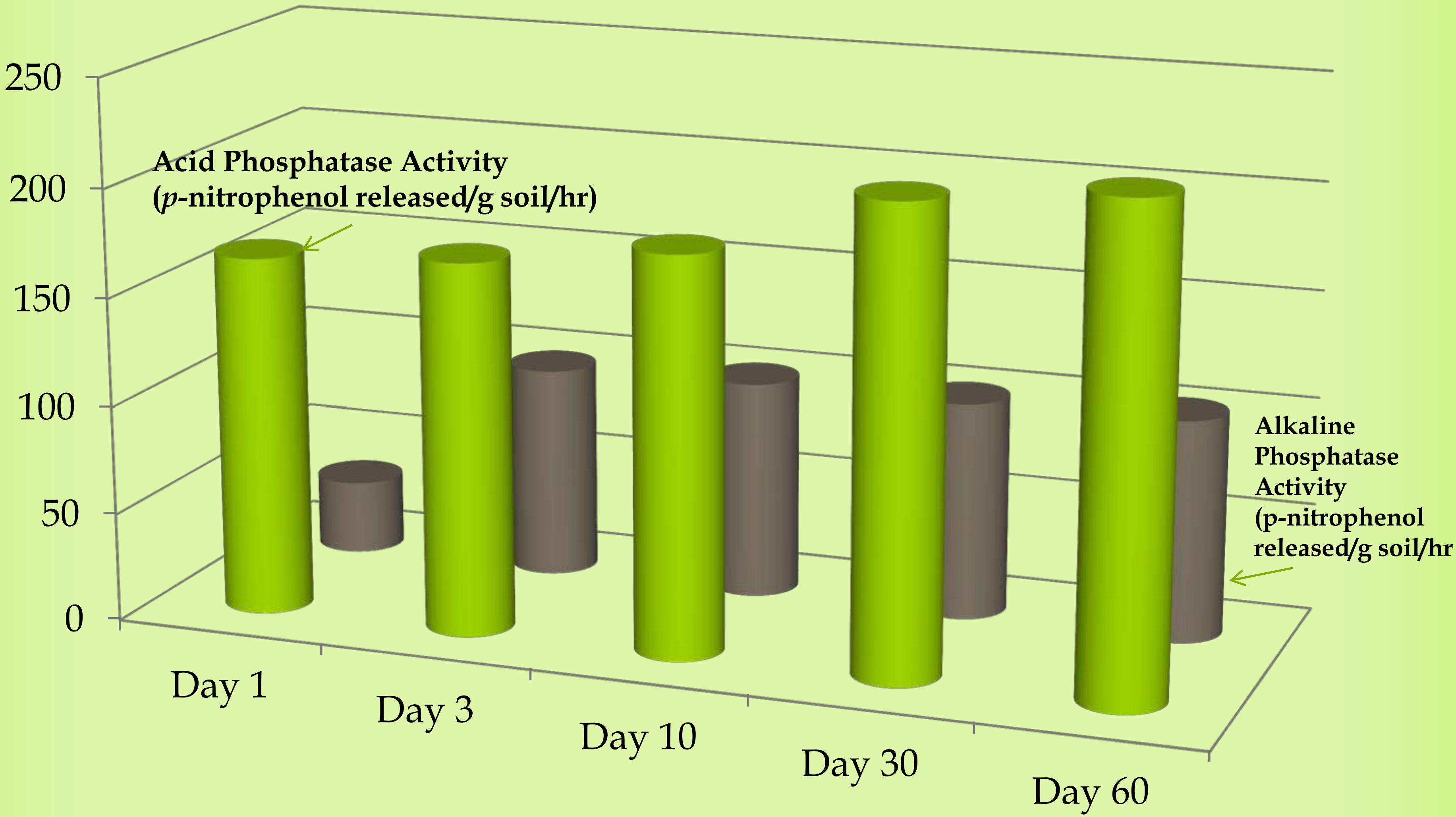
Mean Acid Phosphatase Activities

(*p*-nitrophenol released/g soil/hr)

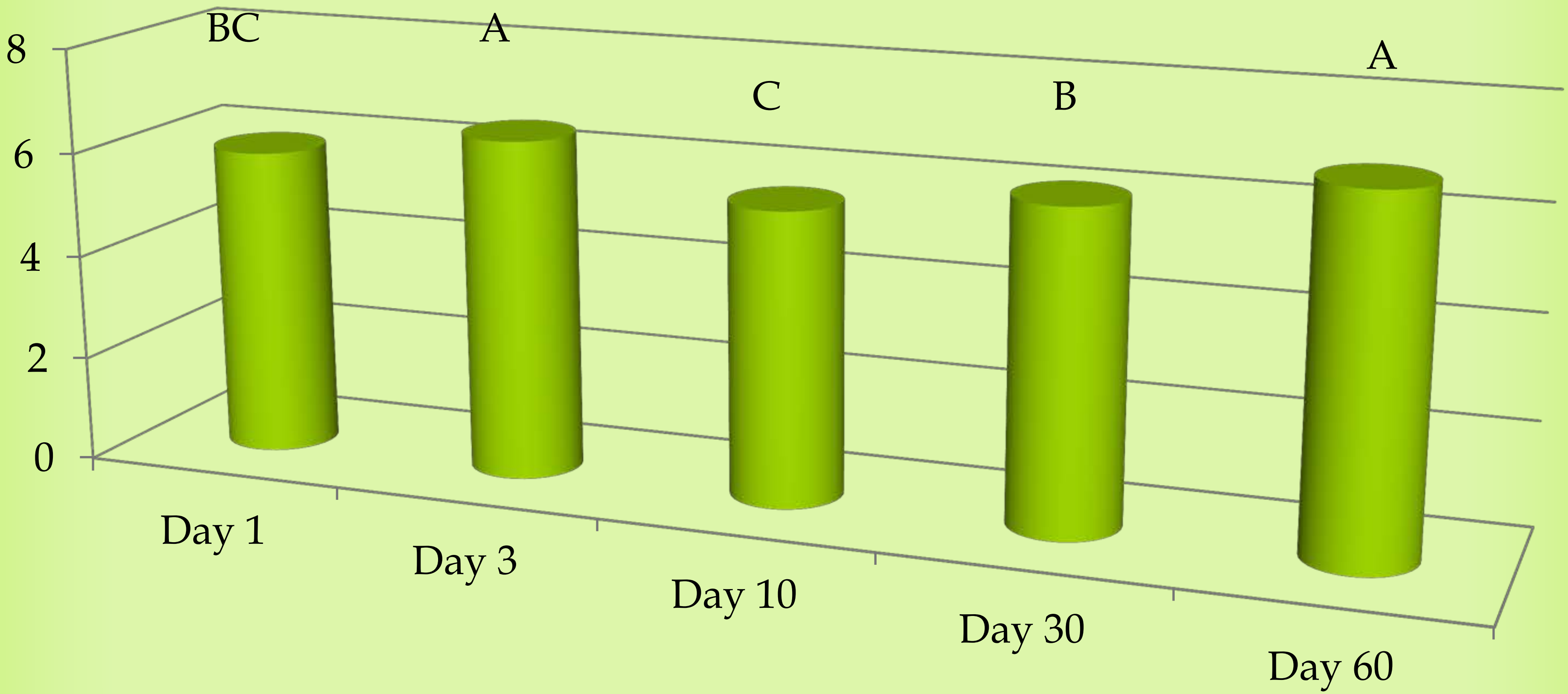


Mean Alkaline Phosphatase Activities
(*p*-nitrophenol released/g soil/hr)

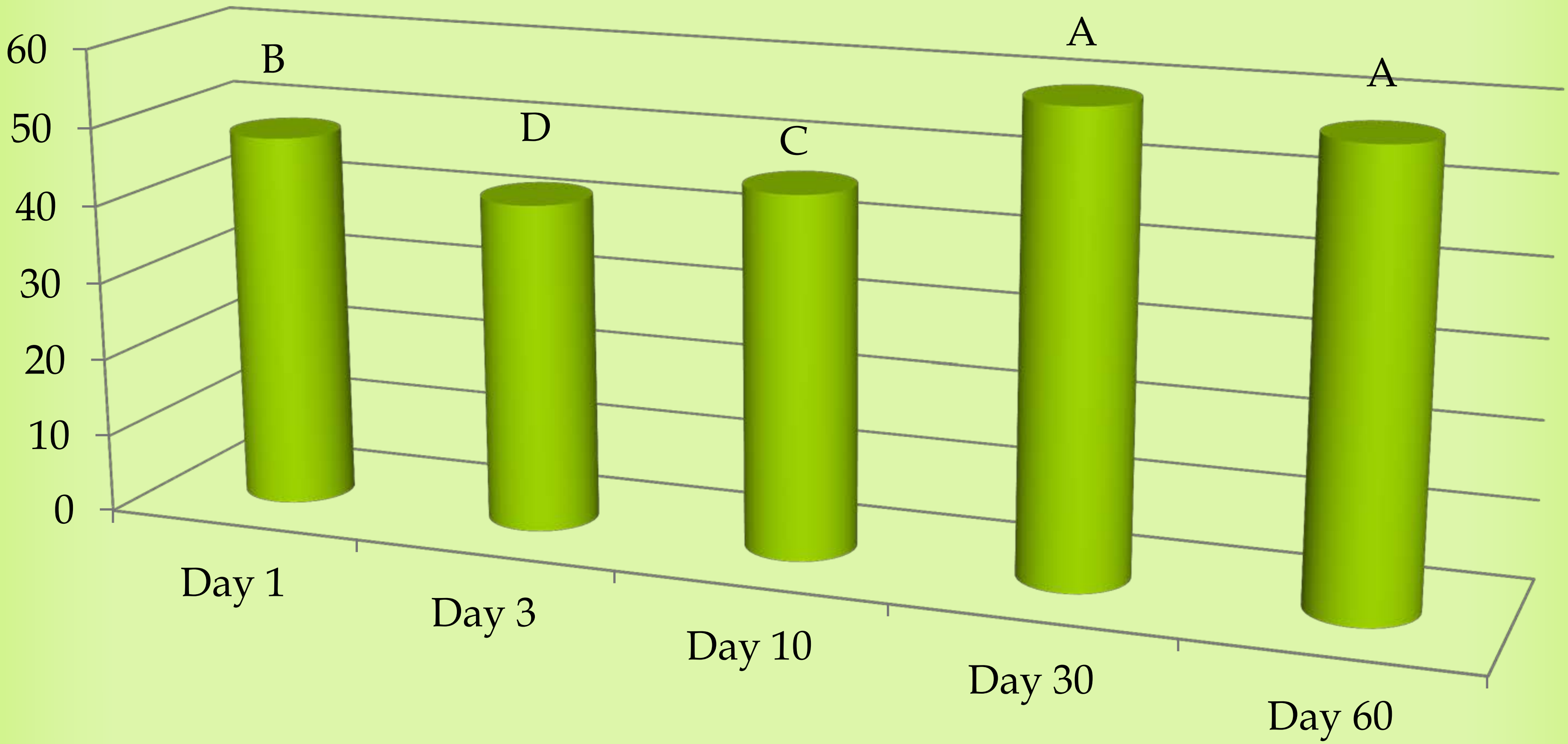



250
200
150
100
50
0
Acid Phosphatase Activity
(p-nitrophenol released/g soil/hr)
Alkaline
Phosphatase
Activity
(p-nitrophenol
released/g soil/hr
Day 1
Day 3
Day 10
Day 30
Day 60

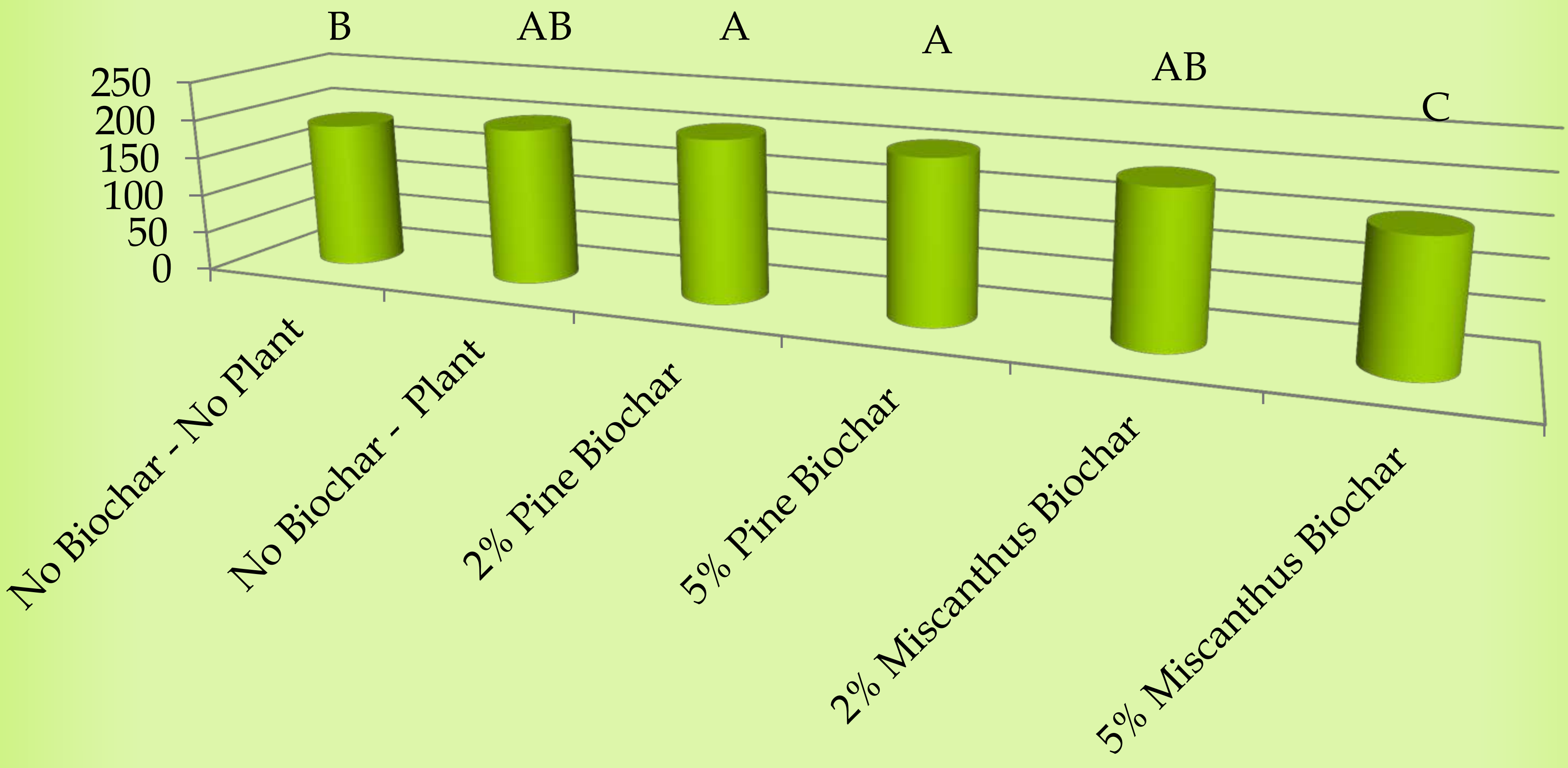
Mean Arylamidase Activities (β-naphthylamine/mL/g soil/hr)



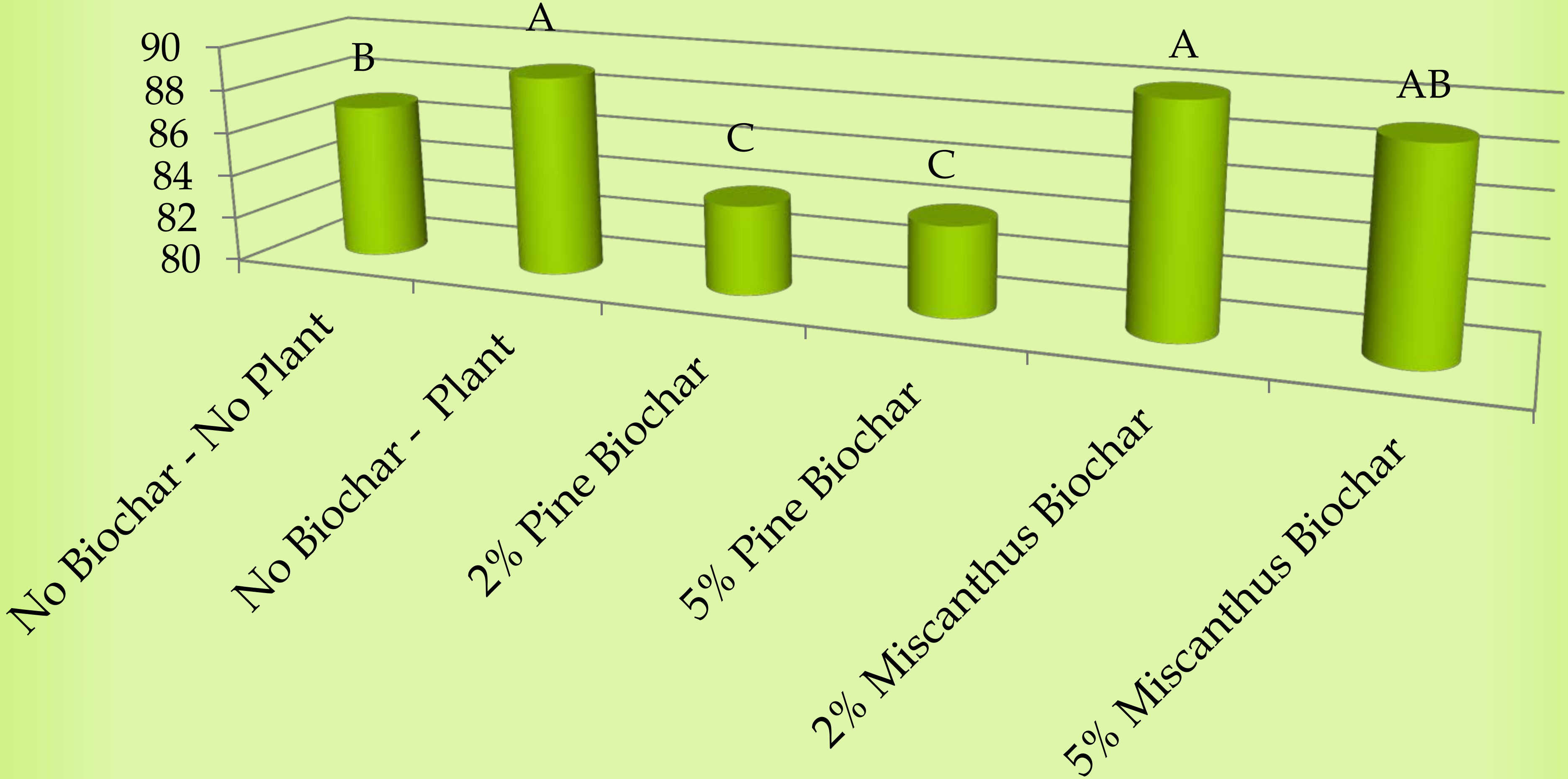
Mean β-Glucosidase Activities (*p*-nitrophenol released g ⁻ soil hr ⁻)

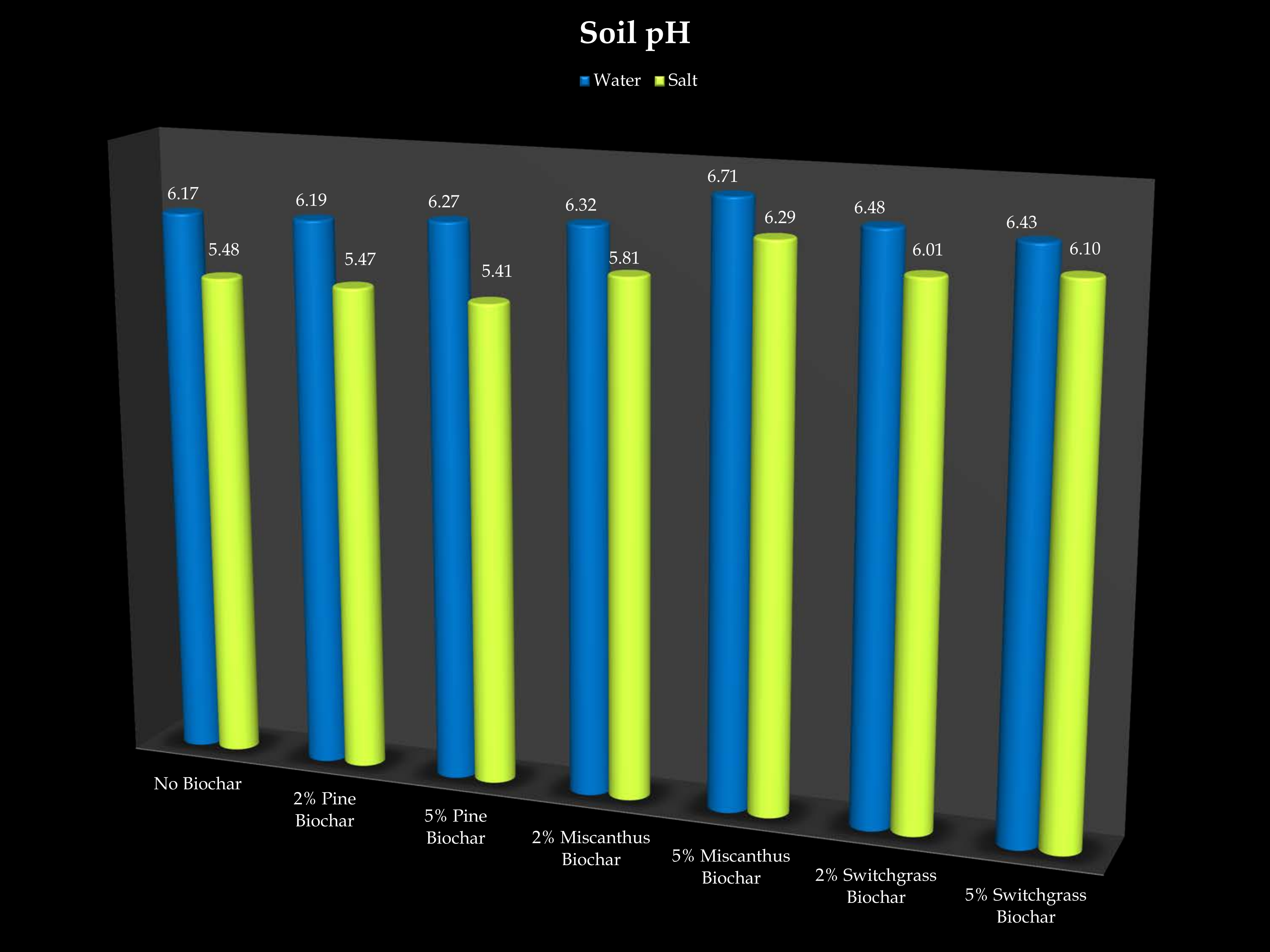


Type of Biochar – Effects on Acid Phosphatase Activity (*p*-nitrophenol released/g soil/hr)

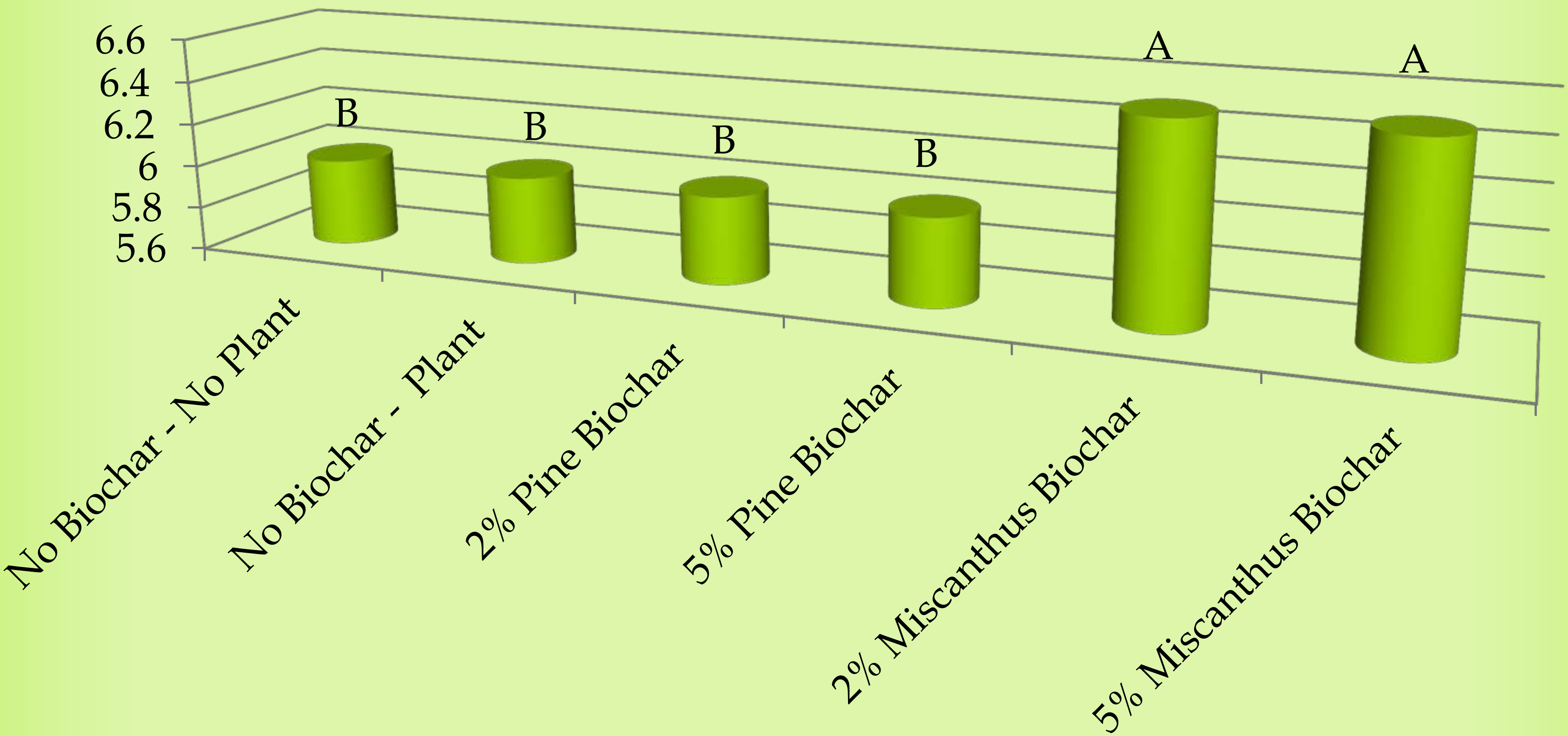


Type of Biochar – Effects on Alkaline Phosphatase Activity (*p*-nitrophenol released/g soil/hr)

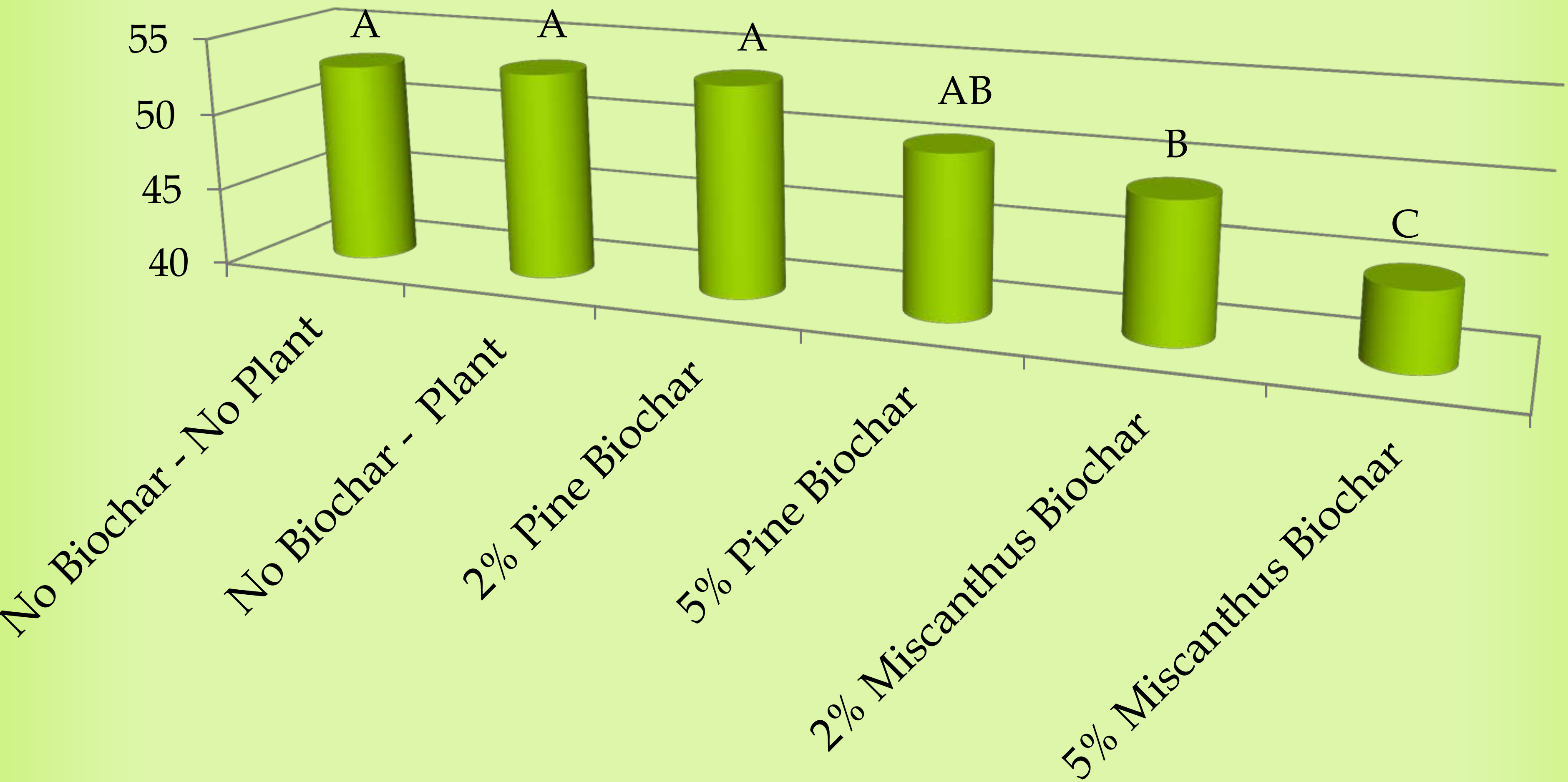



Soil pH
Water
Salt
6.17
5.48
6.19
5.47
6.27
5.41
6.32
5.81
6.71
6.29
6.48
6.01
6.43
6.10
No Biochar
2% Pine Biochar
5% Pine Biochar
2% Miscanthus Biochar
5% Miscanthus Biochar
2% Switchgrass Biochar
5% Switchgrass Biochar

Arylamidase Activity (*p*-nitropheno released/g soil/hr)



β-Glucosidase Activity (*p*-nitrophenol released/g soil/hr)



Conclusions

- Effect of time: The overall activities of enzymes studied in this greenhouse experiment generally increased throughout the course of this study.
- Effect of Biochar type and rates:
  - 1. Biochar type and its application rate affected the activity of acid phosphatase. At 5% miscanthus biochar lowered the activity of this enzyme significantly. At 2% application rate, however, this effect was not significant.

Conclusions
(Continued)

- 2. The pine biochar reduced alkaline phsphatase activities significantly
- 3. The activity of arylamidase increased significantly by the presence of miscanthus biochar regardless of the application rate. The pine biochar, on the other hand, did not affect the activities of this enzyme
- 4. The activity of β-glucosidase was significantly lowered by miscanthus biochar. The inhibition was exacerbated at the higher rate of application but the pine biochar did not affect the activity of this enzyme

Thank you!